Scientific Opinion on application (EFSA-GMO-BE-2011-101) for the placing on the market of herbicide-tolerant genetically modified oilseed rape MON 88302 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto

EFSA Panel

Abstract: Oilseed rape MON 88302 was developed by Agrobacterium tumefaciens-mediated transformation to express the CP4 EPSPS protein, which confers tolerance to glyphosate. The molecular characterisation of oilseed rape MON 88302 did not raise safety issues. Agronomic and phenotypic characteristics of oilseed rape MON 88302 tested under field conditions revealed no biologically relevant differences between oilseed rape MON 88302 and its conventional counterpart, except for days-to-first flowering. No differences in the compositional data requiring further safety assessment were identified. There were no concerns regarding the potential toxicity and allergenicity of the newly expressed CP4 EPSPS protein, and no evidence that the genetic modification might significantly change the overall allergenicity of oilseed rape MON 88302. The nutritional value of oilseed rape MON 88302 is not expected to differ from that of non-GM oilseed rape varieties. There are no indications of an increased likelihood of spread and establishment of feral oilseed rape MON 88302 plants or hybridising wild relatives, unless these plants are exposed to glyphosate. It is unlikely that the observed difference in days-to-first flowering would lead to any relevant increase in persistence or invasiveness. Risks associated with an unlikely, but theoretically possible, horizontal transfer of recombinant genes from oilseed rape MON 88302 to bacteria were not identified. The post-market environmental monitoring plan is in line with the intended uses of oilseed rape MON 88302. In conclusion, the EFSA GMO Panel considers that the information available addresses the scientific requirements of the EFSA GMO Panel and the scientific comments raised by the Member States, and that oilseed rape MON 88302, as described in this application, is as safe as its conventional counterpart and non-GM commercial oilseed rape varieties with respect to potential effects on human and animal health and the environment in the context of the scope of this application.

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SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-BE-2011-101) for the placing on the market of herbicide-tolerant genetically modified oilseed rape MON 88302 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Oilseed rape MON 88302 was developed by *Agrobacterium tumefaciens*-mediated transformation to express the CP4 EPSPS protein, which confers tolerance to glyphosate. The molecular characterisation of oilseed rape MON 88302 did not raise safety issues. Agronomic and phenotypic characteristics of oilseed rape MON 88302 tested under field conditions revealed no biologically relevant differences between oilseed rape MON 88302 and its conventional counterpart, except for days-to-first flowering. No differences in the compositional data requiring further safety assessment were identified. There were no concerns regarding the potential toxicity and allergenicity of the newly expressed CP4 EPSPS protein, and no evidence that the genetic modification might significantly change the overall allergenicity of oilseed rape MON 88302. The nutritional value of oilseed rape MON 88302 is not expected to differ from that of non-GM oilseed rape varieties. There are no indications of an increased likelihood of spread and establishment of feral oilseed rape MON 88302 plants or hybridising wild relatives, unless these plants are exposed to glyphosate. It is unlikely that the observed difference in days-to-first flowering would lead to any relevant increase in persistence or invasiveness. Risks associated with an unlikely, but theoretically possible, horizontal transfer of recombinant genes from oilseed rape MON 88302 to bacteria were not identified. The post-market environmental monitoring plan is in line with the intended uses of oilseed rape MON 88302. In conclusion, the EFSA GMO Panel considers that the information available addresses the scientific requirements of the EFSA GMO Panel and the scientific comments raised by the Member States, and that oilseed rape MON 88302, as described in this application, is as safe as its conventional counterpart and non-GM commercial oilseed rape varieties with respect to potential effects on human and animal health and the environment in the context of the scope of this application.

KEY WORDS

GMO, oilseed rape (*Brassica napus*), MON 88302, herbicide tolerance, CP4 EPSPS, Regulation (EC) No 1829/2003

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[1] On request from the Competent Authority of Belgium for an application (EFSA-GMO-BE-2011-101) submitted by Monsanto, Question No EFSA-Q-2011-01065, adopted on 21 May 2014.

[2] Panel members: Salvatore Arpaia, Andrew Nicholas Edmund Birch, Andrew Chesson, Patrick du Jardin, Achim Gathmann, Jürgen Gropp, Lieve Herman, Hilde-Gunn Hoen-Sorteberg, Huw Jones, József Kiss, Gijs Kleter, Martinus Løvik, Antoine Messéan, Hanspeter Naegeli, Kaare Magne Nielsen, Jaroslava Ovesná, Joe Perry, Nils Rostoks and Christoph Tebbe. Correspondence: gmo@efsa.europa.eu

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SUMMARY

Following the submission of an application (EFSA-GMO-BE-2011-101) under Regulation (EC) No 1829/2003 from Monsanto, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of herbicide-tolerant genetically modified (GM) oilseed rape (*Brassica napus* L.) MON 88302 (Unique Identifier MON-883Ø2-9). The scope of application EFSA-GMO-BE-2011-101 is for import, processing, and food and feed uses of oilseed rape MON 88302 within the European Union (EU) in the same way as any non-GM oilseed rape, but excludes cultivation in the EU.

The EFSA GMO Panel evaluated oilseed rape MON 88302 with reference to the scope and appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed, the environmental risk assessment of GM plants and the post-market environmental monitoring of GM plants. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and analysis of the expression of the corresponding proteins. An evaluation of the comparative analyses of compositional, agronomic and phenotypic characteristics was undertaken, and the safety of the newly expressed proteins and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional wholesomeness. An evaluation of environmental impacts and the post-market environmental monitoring plan was also undertaken.

Oilseed rape MON 88302 was developed by *Agrobacterium tumefaciens*-mediated transformation of the conventional oilseed rape variety Ebony. It expresses 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4, which confers tolerance to the herbicidal active substance glyphosate. The molecular characterisation data established that oilseed rape MON 88302 contains a single insert consisting of the CP4 *epsps* expression cassette. No other parts of the plasmid used for transformation were detected in oilseed rape MON 88302. Bioinformatic analyses and genetic stability studies did not raise safety issues. The levels of the CP4 EPSPS protein in oilseed rape MON 88302 were obtained and reported adequately.

Based on the agronomic and phenotypic characteristics of oilseed rape MON 88302 tested under field conditions, no biologically relevant differences were observed between oilseed rape MON 88302 and its conventional counterpart, except for days-to-first flowering. The observed difference for days-to-first flowering could be attributed either to the variability in the genetic background of the Ebony population or to an unintended effect due to the genetic transformation process. No differences in the compositional data of seeds obtained from oilseed rape MON 88302 requiring further assessment with regard to safety by the EFSA GMO Panel were identified.

The newly expressed CP4 EPSPS protein in oilseed rape MON 88302 is degraded by proteolytic enzymes. The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly expressed CP4 EPSPS protein, and found no evidence that the genetic modification might significantly change the overall allergenicity of oilseed rape MON 88302. As relevant compositional differences were not observed for oilseed rape MON 88302, the nutritional value of food and feed derived from oilseed rape MON 88302 is not expected to differ from that of food and feed derived from non-GM oilseed rape varieties. In addition, the EFSA GMO Panel found no indication that the introduction of the event MON 88302 into other oilseed rape varieties would affect its safety with respect to potential effects on human and animal health.

Application EFSA-GMO-BE-2011-101 covers the import, processing, and food and feed uses of oilseed rape MON 88302, and excludes cultivation. Therefore, the environmental risk assessment is concerned with the accidental release into the environment of viable oilseed rape MON 88302 seeds (i.e. during transport and/or processing), and with the exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals fed GM material and those present in environments exposed to their faecal material (manure and faeces). There is no requirement for scientific information on possible environmental effects associated with the cultivation of oilseed rape MON 88302 in Europe.

The EFSA GMO Panel does not consider the occurrence of occasional feral oilseed rape MON 88302 plants, pollen dispersal and consequent cross-pollination as environmental harm in itself, and is primarily concerned with assessing the environmental consequences of this occurrence on biotic interactions and ecosystems. There are no indications of an increased likelihood of spread and establishment of feral oilseed rape MON 88302 plants in the event of the accidental release into the environment of viable oilseed rape MON 88302 seeds during transport and/or processing, or of hybridising wild relatives that may theoretically have acquired the herbicide tolerance trait through vertical gene flow, unless these plants are exposed to glyphosate-based herbicides. Glyphosate-based herbicides are frequently used for the control of vegetation along railway tracks, on arable land, in open spaces, on pavements and in industrial sites. In these areas, the glyphosate tolerance trait is likely to increase the fitness of GM herbicide-tolerant (GMHT) plants (be it feral plants or progeny from hybrids of oilseed rape and wild relatives) relative to non-glyphosate-tolerant plants when exposed to glyphosate-based herbicides. However, since the occurrence of feral GMHT oilseed rape resulting from seed import spills is likely to be low under an import scenario, feral oilseed rape plants would not create additional agronomic or environmental impacts, even after exposure to glyphosate-based herbicides. The likely effect of the magnitude of the observed difference in days-to-first flowering between oilseed rape MON 88302 and the conventional counterpart on the potential of oilseed rape MON 88302 plants to exhibit increased survival, establishment and fitness is negligible and will thus not lead to any relevant increase in persistence or invasiveness.

Given the scope of this application, only low-level exposure is expected of environmental bacteria, including those in the gastrointestinal tract, to recombinant DNA from oilseed rape MON 88302. Bioinformatic analysis of the inserted DNA and flanking regions did not identify sufficient sequence identity with bacterial DNA (including the modified CP4 *epsps* gene, which has been codon-optimised for expression in plants) that would facilitate homologous recombination-mediated gene transfer between plants and bacteria. Therefore, risks associated with an unlikely, but theoretically possible, horizontal transfer of recombinant genes from oilseed rape MON 88302 to bacteria have not been identified. Considering the scope of this application, the risk to non-target organisms is extremely low owing to the expected confined occurrence of feral oilseed rape plants to ruderal habitats and the low levels of exposure through other routes. In addition, there is no indication that the expression of the CP4 EPSPS protein in glyphosate tolerant plants causes direct adverse effects on non-target organisms. Interactions with the biotic and abiotic environment are therefore not considered a relevant issue.

The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of oilseed rape MON 88302. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the post-market environmental monitoring plan.

In delivering its scientific opinion, the EFSA GMO Panel took into account application EFSA-GMO-BE-2011-101, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. In conclusion, the EFSA GMO Panel considers that the oilseed rape MON 88302, as described in this application, is as safe as its conventional counterpart and non-GM oilseed rape commercial varieties, and is unlikely to have adverse effects on human and animal health and the environment in the context of the scope of this application.

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BACKGROUND

On 8 September 2011, the European Food Safety Authority (EFSA) received from the Belgian Competent Authority an application (Reference EFSA-GMO-BE-2011-101) for authorisation of GM oilseed rape MON 88302 (Unique Identifier MON-883Ø2-9), submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on GM food and feed[4].

After receiving the application EFSA-GMO-BE-2011-101 and in accordance with Articles 5(2)(b) and 17(2)(b) of the Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission, and made the summary of the application publicly available on the EFSA website[5]. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of the Regulation (EC) No 1829/2003. On 13 February 2012 and 13 March 2012, EFSA received additional information requested under completeness check (19 October 2011 and 2 March 2012, respectively). On 2 April 2012, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission, and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[6] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had three months after the date of receipt of the valid application (until 2 July 2012) to make their opinion known.

The EFSA GMO Panel carried out an evaluation of the scientific risk assessment of oilseed rape MON 88302 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The EFSA GMO Panel took into account the appropriate principles described in its guidelines for the the risk assessment of GM plants and derived food and feed (EFSA GMO Panel, 2011a), the environmental risk assessment of GM plants (EFSA GMO Panel, 2010c) and on the post-market environmental monitoring of GM plants (EFSA GMO Panel, 2011b). Furthermore, the EFSA GMO Panel also took into consideration the scientific comments of Member States, the additional information provided by the applicant and the relevant scientific publications.

On 13 September 2012, 30 January 2013, 13 August 2013 and 12 December 2013, the EFSA GMO Panel requested additional information from the applicant. The applicant provided the requested information on 10 October 2012, 27 February 2013, 2 September 2013 and 3 March 2014, respectively. The applicant also spontaneously provided additional information on 19 December 2013. After evaluation of the full data package, the EFSA GMO Panel finalised its risk assessment of oilseed rape MON 88302.

In giving its scientific opinion on oilseed rape MON 88302 to the European Commission, Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of six months from the acknowledgement of the valid application. As additional information was requested by the EFSA GMO Panel, the time limit of six months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(5).

[4] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

[5] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2011-01065

[6] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific assessment of oilseed rape MON 88302 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003.

Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)(e) of Regulation (EC) No 1829/2003.

The EFSA GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the EFSA GMO Panel did not consider proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

ASSESSMENT

1. Introduction

Oilseed rape MON 88302 (Unique Identifier MON-883Ø2-9) is assessed with reference to its intended uses and the appropriate principles described in the applicable guidelines of EFSA's Scientific Panel on GMOs (EFSA GMO Panel, 2010c, 2011a, b). The risk assessment presented here is based on the information provided in the application relating to oilseed rape MON 88302 submitted in the European Union (EU), scientific comments raised by Member States and relevant scientific publications.

The scope of application EFSA-GMO-BE-2011-101 is for import, processing and food and feed uses of oilseed rape MON 88302 and does not include cultivation in the EU. Thus, oilseed rape MON 88302 will be imported into the EU for food or feed uses in the same way as any commercial oilseed rape variety.

The main commodity of oilseed rape is oil, which is used for a variety of foods, including frying and baking oils, salad oils, margarines and shortenings. Solid meal left after oil extraction of oilseed rape can be used as animal feed.

Oilseed rape MON 88302 was developed to confer tolerance to the herbicidal active substance glyphosate up to the flowering stage of plant development[7]. Tolerance to glyphosate (N-(phosphonomethyl)glycine) was achieved by the expression of 5-enolpyruvyl-shikimate-3-phosphate synthase (CP4 EPSPS), which has a low binding affinity for glyphosate and maintains enzymatic activity in its presence. The genetic modification in oilseed rape MON 88302 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, processing characteristics and overall use of oilseed rape as a crop.

2. Issues raised by the Member States

The comments raised by Member States are addressed in Annex G of the EFSA overall opinion[8] and were taken into consideration during the evaluation of the risk assessment.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs

Oilseed rape MON 88302 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation. Hypocotyl segments of oilseed rape variety Ebony were co-cultured with a disarmed *A. tumefaciens* strain ABI containing the vector PV-BNHT2672[9].

The PV-BNHT2672 vector includes one T-DNA, containing the CP4 *epsps* expression cassette[10], which confers tolerance to glyphosate. The CP4 *epsps* cassette contains the following genetic elements: the *FMV/Tsf1* chimeric promoter, as a combination of the enhancer sequences from the *Figwort Mosaic Virus 35S* RNA with the promoter from the *Tsf1* gene of *Arabidopsis thaliana*; the *Tsf1* leader sequence from *A. thaliana*; the *CTP2* target sequence of the *shkG* gene, from *A. thaliana*; the codon-optimised CP4 *epsps* sequence of the *aroA* gene from *Agrobacterium* sp. strain CP4; and the 3′ untranslated region of the ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit (*rbcS2*) *E9* gene, from *Pisum sativum*.

[7] Part II Scientific information, Section A.2.2.1.
[8] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2011-01065
[9] Part II Scientific information, Section A.2.1.1.
[10] Part II Scientific information, Section A.2.1.2.

The vector backbone sequence contains: the aminoglycoside adenyltransferase (*aadA*) gene from transposon Tn*7* conferring resistance to the antibiotics streptomycin and spectinomycin; the origin of replication (*oriV*) from broad host plasmid RK2; the *ori pBR322* from plasmid vector pBR322; and the *rop* sequence from *Escherichia coli*.

3.1.2. Transgene constructs in the GM plant

Molecular characterisation of oilseed rape MON 88302 was performed by Southern analysis, polymerase chain reaction (PCR) and DNA sequence analysis, in order to determine insert copy number, size and organisation of the inserted sequences and to confirm the absence of plasmid backbone sequences[11]. The approach used was acceptable in terms of both coverage and sensitivity.

Southern analyses indicated that the oilseed rape MON 88302 contains a single insert, which consists of a single copy of the T-DNA in the same configuration as in the PV-BNHT2672 transformation vector. The insert and copy number were confirmed by the hybridisation signals generated with three restriction enzymes, together with three overlapping T-DNA probes. The absence of vector backbone sequences was confirmed by Southern analysis, using the same restriction enzymes with three backbone-specific overlapping probes[12].

The insert and 5′ and 3′ flanking regions of oilseed rape MON 88302 were sequenced. The results were in line with those shown by the Southern analyses. The insert of 4 428 bp is identical to the T-DNA of PV-BNHT2672. As a result of the transformation, a 9 bp insertion and a 29 bp deletion of plant DNA occurred adjacent to the 3′ end of the MON 88302 insert[13]. A single nucleotide difference between the sequence of the pre-insertion site of the conventional counterpart and the genomic DNA sequence flanking the 3′ end of the MON 88302 insert was also identified. The possible interruption of known endogenous oilseed rape genes by the insertion in oilseed rape MON 88302 was evaluated by bioinformatic analyses of the pre-insertion locus and of the genomic sequences flanking the insert[14]. Data show that the insertion site of the event MON 88302 is located in a ~ 90 nucleotide intergenic region between the 3′ ends of two likely transcriptional elements. However, there is no indication that either of the two transcriptional elements in oilseed rape MON 88302 was disrupted. The results of segregation and bioinformatic analyses established that the insert is located in the nuclear genome.

In order to assess whether the open reading frames (ORFs) present within the insert and spanning the junction sites raise any safety issues, their putative translation products were compared for similarities to known allergens and toxins by using suitable algorithms and appropriate databases[15]. None of the ORF-derived amino acid sequences identified at the junctions and in the inserted sequences showed significant similarities with known toxins or allergens. These bioinformatic analyses support the conclusion that, even in the unlikely event that any of the new ORFs at the junctions were translated, they would not raise a safety issue.

In order to conclude on the possibility of horizontal gene transfer by homologous recombination (HR), the applicant performed sequence similarity searches between the MON 88302 insert (including the flanking sequences) and bacteria and plasmid sequence databases.

A single alignment with *A. tumefaciens* Ti-plasmid sequence of sufficient length and identity for HR (de Vries and Wackernagel, 2002; Monier et al, 2007; Hülter and Wackernagel, 2008; EFSA, 2009b; Overballe-Petersen et al, 2013) was identified, as expected. The alignment displayed 99.3 % identity over a 296 bp fragment of the Ti-plasmid containing the left border region used for transfer of the T-DNA. As there is no other identity with Ti-sequences of sufficient length, there is no indication for a possible double HR with the Ti-plasmid of *A. tumefaciens*.

[11] Part II Scientific information, Section A.2.2.2.
[12] Part II Scientific information, Section A.2.2.2.(ii).
[13] Part II Scientific information, Section A.2.2.2.(iii).
[14] Part II Scientific information, Section A.2.2.2.(v).
[15] Part II Scientific information, Section A.2.2.2.(v).

High sequence similarity with other bacterial DNA sequences which would trigger further consideration of HR-facilitated gene transfer was not identified. The CP4 *epsps* gene is of bacterial origin, but the plant-inserted version has extensive changes in its nucleotide sequence as a result of optimisation of the codon usage for expression in plant cells. These sequence changes have lowered the overall sequence similarity to the bacterial counterpart to levels that are considered insufficient (EFSA, 2009b) for HR in bacteria.

The likelihood and potential consequences of the plant-to-bacteria gene transfer are described in Section 6.1.1.2.

3.1.3. Information on the expression of the insert

Levels of the CP4 EPSPS protein were assessed in a range of tissues (root, leaf, forage and seed) and developmental stages of oilseed rape MON 88302 by enzyme-linked immunosorbent assay (ELISA). Material was harvested in 2009 from three locations in the USA and three locations in Canada. Samples analysed included those treated and those not treated with glyphosate[16]. Considering the scope of the application, the CP4 EPSPS protein levels in seeds are considered the most relevant. Values from all six field trials had an arithmetic mean of 31 µg/g dry weight (with standard error 1.4 and range 22–42 µg/g) for untreated plots and of 27 µg/g dry weight (with standard error 1.3 and range 22–46 µg/g) for treated plots. These results indicated that glyphosate treatment had no significant effect on the levels of the newly expressed proteins in seeds.

3.1.4. Inheritance and stability of inserted DNA

Genetic stability of the oilseed rape MON 88302 insert was assessed by Southern analysis of genomic DNA from four consecutive generations. The restriction enzyme/probe combinations used were sufficient to conclude that all the plants tested retained the single copy of the insert and flanking regions, which were stably inherited in subsequent generations[17].

Phenotypic stability was observed by segregation analysis of the herbicide tolerance trait in plants from three generations of oilseed rape MON 88302. The results supported the presence of a single insertion, segregating in a Mendelian fashion.

3.2. Conclusion

The molecular characterisation data establish that oilseed rape MON 88302 contains a single insert consisting of one copy of the CP4 *epsps* expression cassette. Bioinformatic analyses of the ORFs spanning the junction sites within the insert or between the insert and genomic DNA did not raise safety issues. Bioinformatic analyses also indicated that the potential for HR with bacterial DNA was very low. The stability of the inserted DNA and of the introduced herbicide tolerance trait was confirmed over several generations. Protein levels were obtained and reported adequately. The potential impacts of the CP4 EPSPS protein levels, quantified in field trials carried out in the USA and Canada, are assessed in the sections on food/feed safety assessment (Section 5) and environmental risk assessment (Section 6).

4. Comparative analysis

4.1. Evaluation of relevant scientific data

The applicant performed the comparative assessment using the most recent statistical methodology recommended by the EFSA GMO Panel (EFSA GMO Panel, 2010a, 2011a). This recommends the simultaneous application of a test of difference to determine whether the GM plant is different from its conventional counterpart, and a test of equivalence to determine whether the GM plant falls within the natural variation estimated from the non-GM oilseed rape reference varieties included in the study. As described by EFSA (EFSA GMO Panel 2011a), the result of the equivalence test is categorised into

[16] Part II Scientific information, Section A.2.2.3.
[17] Part II Scientific information, Section A.2.2.4.

four possible outcomes to facilitate the drawing of conclusions with respect to the presence or absence of equivalence. These four categories are: category I, indicating full equivalence; category II, indicating that equivalence is more likely than non-equivalence; category III, indicating that non-equivalence is more likely than equivalence; and category IV, indicating non-equivalence.

4.1.1. Choice of comparator[18]

Application EFSA-GMO-BE-2011-101 presents data on agronomic and phenotypic characteristics, as well as seed composition of oilseed rape MON 88302 derived from field trials performed in the USA and Canada in 2009 and in Chile in 2009/2010 (Table 1). In addition, seed and pollen characteristics of oilseed rape MON 88302 were evaluated under laboratory (growth chamber) conditions.

The non-GM oilseed rape variety Ebony was used as comparator in the agronomic and phenotypic field trials, the seed germination tests, the pollen morphology tests and the compositional studies supplied by the applicant (Table 1). Ebony was the oilseed rape variety originally transformed to establish the transformation event MON 88302. The EFSA GMO Panel considers that Ebony has a genetic background comparable to that of oilseed rape MON 88302 used in these studies, as documented by the pedigree[19]. Therefore, Ebony is regarded as an appropriate conventional counterpart.

Table 1: Overview of comparative assessment studies with oilseed rape MON 88302 provided with application EFSA-GMO-BE-2011-101

Study focus	Endpoints	Study details	Comparators		Non-genetically modified oilseed rape reference varieties
			Conventional counterpart	Negative segregant	
Agronomic and phenotypic characteristics and/or composition of harvested seeds tested under field conditions	Various endpoints (see Sections 4.1.2.1 and 4.1.2.2)	2009, North America (two locations in the USA and three in Canada)[20]	1 (Ebony)	0	7
		2009/2010, Chile (four locations)[21]	1 (Ebony)	0	12
	Days-to-first flowering	2010, North America (three locations in the USA and three in Canada)[22]	1 (Ebony)	1	20
Agronomic and phenotypic characteristics tested under controlled conditions	Seed characteristics	Seeds collected from field trials performed in 2009 in the USA[23] (a)	1 (Ebony)	0	2 (b)
	Pollen characteristics	Pollen collected from plants grown under growth chamber conditions[24]	1 (Ebony)	0	2 (b)

(a): Field trial was exclusively conducted for agronomic and phenotypic characteristics.
(b): In addition, two genetically modified varieties were also included.

[18] Part II Scientific information, Section A.3.1-2.
[19] Part II Scientific information, Section A.2.2.
[20] Part II Scientific information, Section A.3.4. Annex: McPherson and Ahmad (2012a).
[21] Part II Scientific information, Section A.3.4. Annex: McPherson and Ahmad (2012a).
[22] Additional information received on 10/10/2012. Annex: McPherson (2012).
[23] Part II Scientific information, Section A.3.4. Annexes: McPherson (2010a, 2011a).
[24] Part II Scientific information, Section A.3.4. Annexes: McPherson (2010b, 2011b).

4.1.2. Agronomic and phenotypic characteristics[25]

4.1.2.1. Agronomic and phenotypic characteristics tested under field conditions

Field trials for the agronomic and phenotypic assessment of oilseed rape MON 88302 were conducted in the oilseed rape growing areas in North America during the 2009 growing season (two locations in the USA (one in Minnesota and one in North Dakota); three locations in Canada (two in Manitoba and one in Saskatchewan)) and in Chile during the 2009/2010 growing season (four locations: two in Maipo and two in Cachapoal). At each site, the following materials were grown in a randomised complete block design with four replicates: oilseed rape MON 88302, the conventional counterpart (Ebony) and four different non-GM oilseed rape reference varieties, all treated with the required maintenance pesticides (including conventional herbicides); and oilseed rape MON 88302 treated with glyphosate and the required maintenance pesticides (including conventional herbicides). In total, 12 non-GM oilseed rape reference varieties were included in the 2009 and 2009/2010 agronomic and phenotypic field trials (Table 1).

Ten endpoints were measured in the 2009 and 2009/2010 agronomic and phenotypic field trials: early stand count, days-to-first flowering, seed maturity, lodging, plant height, pod shattering, seed moisture, seed quality, yield and final stand count. Visually observable responses to naturally occurring diseases, abiotic stress and arthropod damage were also recorded in order to provide indications of altered stress responses of oilseed rape MON 88302 compared with its conventional counterpart.

Statistically significant differences between oilseed rape MON 88302 and its conventional counterpart (Ebony) were observed for three endpoints: seed maturity, lodging and days-to-first flowering.

- For the seed maturity and lodging endpoints, the test of equivalence indicated equivalence to the non-GM oilseed rape reference varieties (equivalence category I). The observed differences did not require further assessment.

- For the days-to-first flowering endpoint, the test of equivalence indicated non-equivalence to the non-GM oilseed rape reference varieties (equivalence category IV) when oilseed rape MON 88302 was treated with the intended herbicide and also when it was treated with maintenance pesticides only. The number of days-to-first flowering was consistently higher in oilseed rape MON 88302 (first flowering occurred approximately five days later) than in the conventional counterpart and non-GM oilseed rape reference varieties. This observed difference was further assessed.

  Following a request of the EFSA GMO Panel for more data on the difference observed in days-to-first flowering, the applicant supplied agronomic and phenotypic data from additional field trials performed in 2010 in the USA (three locations) and Canada (three locations) (Table 1). In these field trials, oilseed rape MON 88302 was grown together with its conventional counterpart (Ebony) and 20 non-GM oilseed rape reference varieties. At each site, oilseed rape MON 88302, its conventional counterpart and four non-GM oilseed reference varieties treated with maintenance pesticides (including conventional herbicides) were grown in a randomised complete block design with four replications. The applicant used a negative segregant of oilseed rape MON 88302 as a supplementary comparator, in addition to Ebony, in order to investigate whether the increase in days-to-first flowering between oilseed rape MON 88302 and the conventional counterpart was likely to be due to a trait effect or the variability in the genetic background of the Ebony population. As it is rare for any oilseed rape variety including Ebony to be 100 % inbred and homozygous at every locus, the individual oilseed rape plant used for the genetic transformation could have differences compared with the majority of the other individuals in the Ebony population. Therefore, one

[25] Part II Scientific information, Sections A.3.1 and A.3.4, and additional information received on 10/10/2012 and 27/02/2013. Annex: McPherson and Ahmad (2012a).

justification for the use of a negative segregant is that it might be closer to the genetic background of oilseed rape MON 88302 than the progeny of the individual that was selected from the Ebony population as the conventional counterpart.

Whereas a difference in days-to-first flowering was observed between oilseed rape MON 88302 and the conventional counterpart, no difference was observed between oilseed rape MON 88302 and the negative segregant. This suggests that the observed difference between oilseed rape MON 88302 and the conventional counterpart does not necessarily indicate a potential unintended effect associated with the presence of the trait. It may be attributed to either the variability in the genetic background of the Ebony population or an unintended effect due to the genetic transformation process producing oilseed rape MON 88302. Based on the dataset provided by the applicant, the EFSA GMO Panel cannot rule out the possibility that the genetic transformation process establishing oilseed rape MON 88302 resulted in an unintended effect influencing days-to-first flowering. The potential consequences of this observed difference are discussed further in Section 6.

There was no statistically significant difference between oilseed rape MON 88302 and its conventional counterpart for the remaining agronomic and phenotypic endpoints. Additionally, no altered stress responses of oilseed rape MON 88302 were observed compared with its conventional counterpart with regard to visually observable response to naturally occurring diseases, abiotic stress and arthropod damage.

4.1.2.2. Agronomic and phenotypic characteristics tested under controlled conditions

(a) Seed characteristics

The applicant also reported data on seed characteristics of oilseed rape MON 88302. Seed germination tests with seeds harvested from oilseed rape MON 88302, its conventional counterpart (Ebony) and two non-GM oilseed reference varieties, grown under field conditions in North Dakota (USA) in 2009, were performed to evaluate seed characteristics under growth chamber conditions. Seeds were incubated in growth chambers under controlled conditions at different temperatures. The endpoints analysed were the numbers of germinated seeds, dead seeds and dormant seeds. The applicant found no statistically significant differences between oilseed rape MON 88302 and its conventional counterpart.

Although the applicant refers to seed dormancy when discussing the generated data on seed characteristics of oilseed rape MON 88302, no data on secondary seed dormancy, induced in controlled conditions, were supplied. Therefore, the EFSA GMO Panel considers that only the conclusions on seed germination of oilseed rape MON 88302 are substantiated by the provided data.

(b) Pollen characteristics

The applicant also reported data on pollen characteristics of oilseed rape MON 88302. Pollen morphology and viability from oilseed rape MON 88302, its conventional counterpart (Ebony) and two non-GM oilseed rape reference varieties were measured. Pollen was obtained from plants grown in pots under growth chamber conditions. Parameters analysed were pollen viability, diameter and general morphology. The applicant observed no significant differences between oilseed rape MON 88302 and its conventional counterpart for any of these parameters.

Owing to the insensitivity of the Alexander's stain technique to measure pollen viability, the EFSA GMO Panel considers the data on pollen viability inappropriate for the comparative assessment. Alexander's stain is a preliminary test assessing pollen grain maturity, but does not directly measure pollen viability or germination capacity (Dafni, 1992).

4.1.3. Compositional analysis[26]

The field trials to produce material for the comparative compositional assessment of oilseed rape MON 88302 are described in Table 1 (see Section 4.1.2.1).

The compositional analysis of oilseed rape seeds was carried out according to OECD recommendations (OECD, 2001). Oilseed rape seeds were harvested and analysed for proximates (protein, total fat, ash, moisture and carbohydrate), fibre fractions (acid detergent fibre (ADF), neutral detergent fibre (NDF) and total detergent fibre (TDF)), 18 amino acids, 14 fatty acids, vitamin E (α-tocopherol) and anti-nutrients (i.e. phytic acid, total tannins, alkyl glucosinolates, indolyl glucosinolates, total glucosinolates, sinapic acid and sinapine). Forage was not analysed, since oilseed rape forage is rarely consumed by animals and its analysis is not recommended by OECD. In total, 67 parameters were analysed in seeds from the North American field trials and 63 from the South American field trials. In 15[27] of these parameters, more than 50 % of the observations, which were below the limit of quantification, were excluded from the analysis[28]. In total, 52 analytes were available for statistical analysis.

The test of difference on chemical analytical data from seeds of plants sprayed with maintenance pesticides identified statistically significant differences between oilseed rape MON 88302 and its conventional counterpart for 14 parameters (the proximates carbohydrates, ash and total fat; the amino acid lysine; the fatty acids 16:0 palmitic acid, 18:0 stearic acid, 18:1 oleic acid, 18:2 linoleic acid, 18:3 linolenic acid, 20:0 arachidic acid, 20:1 eicosenoic acid and 22:0 behenic acid; the mineral calcium; and vitamin E). No differences were identified for the anti-nutrients. The test of equivalence indicated equivalence or that equivalence was more likely than not (equivalence categories I and II; EFSA GMO Panel, 2011a) for all analytes. Within equivalence category II, two parameters (vitamin E and calcium) fell into outcome type 4 (EFSA GMO Panel, 2011a). Given the magnitude of these changes and the characteristics of these endpoints, the EFSA GMO Panel concludes that the differences do not raise safety concerns for humans and animals. The equivalence test could not be performed on moisture because of a lack of variation in the moisture parameter among the non-GM oilseed rape reference varieties prohibited an estimation of equivalence limits.

The test of difference in the compositional data on seeds of oilseed rape MON 88302 treated with glyphosate in addition to required maintenance pesticides, and data on the conventional counterpart treated only with required maintenance pesticides, identified statistically significant differences for 17 parameters (the proximates carbohydrates, ash and total fat; the amino acid lysine; the fatty acids 16:0 palmitic acid, 18:0 stearic acid, 18:1 oleic acid, 18:2 linoleic acid, 18:3 linolenic acid, 20:0 arachidic acid, 20:1 eicosenoic acid and 22:0 behenic acid; the minerals calcium, copper and zinc; vitamin E; and the anti-nutrient sinapine). The test of equivalence indicated equivalence or that equivalence was more likely than not (equivalence categories I and II; EFSA GMO Panel, 2011a) to the non-GM oilseed rape reference varieties for all analytes. An equivalence test could not be performed on moisture owing to a lack of variation among the non-GM reference varieties. Within the equivalence category II, two parameters (vitamin E and calcium) fell into outcome type 4 (EFSA GMO Panel, 2011a). Given the magnitude of these changes and the characteristics of these endpoints, the EFSA GMO Panel concludes that the differences do not raise safety concerns for humans and animals.

The EFSA GMO Panel considered the total set of compositional data supplied and the outcome of the statistical analysis comparing oilseed rape MON 88302, its conventional counterpart and the set of non-GM oilseed rape varieties with information available in the scientific literature. The EFSA GMO

[26] Part II Scientific information, Section A.3.3.

[27] Nineteen in the seed material from the South American field trials.

[28] Compounds being below the limit of quantification were caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), heptadecanoic acid (17:0), heptadecenoic acid (C17:1), gamma linolenic acid (C18:3), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4), eicosapentaenoic acid (C20:5), erucic acid (C22:1), docosapentaenoic acid (C22:5), docosahexaenoic acid (C22:6), nervonic acid (C24:1) and sodium.

Panel concludes that the compositional data give no indication that the genetic modification induced unintended effects for which further assessment was needed.

4.2. Conclusion

Based on the agronomic and phenotypic characteristics of oilseed rape MON 88302 tested under field conditions, no biologically relevant differences were observed between oilseed rape MON 88302 and its conventional counterpart, except for days-to-first flowering, which needs further assessment (Section 6). The observed difference for days-to-first flowering could be attributed to either the variability in the genetic background of the Ebony population or an unintended effect due to the genetic transformation process.

No biologically relevant differences were identified in the compositional characteristics of seeds obtained from oilseed rape MON 88302 that would require further assessment with regard to safety.

5. Food/feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Effect of processing[29]

Oilseed rape MON 88302 will be used for the production and manufacture of food and feed products, in the same way as any other commercial oilseed rape variety. Based on the outcome of the comparative assessment, the effect of processing on oilseed rape MON 88302 is not expected to be different from that on conventional oilseed rape.

The EFSA GMO Panel considers it unlikely that the CP4 EPSPS enzyme activity would remain in the processed products derived from oilseed rape MON 88302 (see Section 5.1.2.1).

5.1.2. Toxicology[30]

The CP4 EPSPS protein is the only newly expressed protein in oilseed rape MON 88302. The EFSA GMO Panel performed the assessment of this protein considering its identity, its functional properties and bioinformatics studies on its amino acid sequence. Previous EFSA GMO Panel assessments of the CP4 EPSPS protein newly expressed in other GM plants were also considered.

The outcome of the molecular characterisation and the comparative analysis of oilseed rape MON 88302 did not identify issues requiring further toxicological assessment.

5.1.2.1. Protein used for safety assessment

The CP4 EPSPS protein derived from MON 88302 seeds was characterised by N-terminal sequence and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) analysis and several other assays. The protein was 99 % pure and recognised by a specific antibody in Western blot analysis. Sequencing of the seed-produced CP4 EPSPS showed the expected 15 amino acids at its N-terminal[31]. MALDI-TOF-MS analysis showed a high coverage (85.5 %) of the expected protein sequence. The average mass of the seed-produced CP4 EPSPS protein was 47.4 kDa, which is consistent with the theoretical mass of the CP4 EPSPS protein starting at position 2. The seed-produced CP4 EPSPS protein was not glycosylated, and remained functional in enzymatic assay.

[29] Part II Scientific information, Section A.3.5.
[30] Part II Scientific information, Section A.4.
[31] Part II Scientific information. Annex: Bhatka (2010).

Given the low levels of the CP4 EPSPS protein expressed in oilseed rape MON 88302, the recombinant protein produced in an *E. coli* strain transformed with plasmid pMON 21104[32] was used for safety testing.

The equivalence between the *E. coli*-produced and the plant-derived proteins in terms of immunological properties, apparent molecular weight and function was determined by Western blot, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), glycosylation and enzymatic activity, respectively. Based on the outcomes of these tests, the EFSA GMO Panel accepts the use of CP4 EPSPS produced by *E. coli* as an appropriate substitute test material for the CP4 EPSPS protein present in oilseed rape MON 88302. The *E. coli*-produced CP4 EPSPS protein was functionally inactivated at 75 °C in 15 minutes; furthermore, it was shown by SDS-PAGE that there was no production of protein fragments from heat-treated samples after incubation for 15 or 30 minutes up to 95 ° C[33].

5.1.2.2. Toxicological assessment of the newly expressed protein

The EFSA GMO Panel has previously assessed the CP4 EPSPS protein in the context of several applications for the placing on the EU market of GM crops, including a GM oilseed rape (EFSA, 2004; EFSA GMO Panel 2009, 2012, 2013a), and other GM plants (including soybean, EFSA, 2008; EFSA GMO Panel 2012a, b; maize, EFSA, 2007, 2009a; EFSA GMO Panel 2011c; and cotton, EFSA GMO Panel, 2013d). Updated bioinformatic analysis[34] of the amino acid sequence of the CP4 EPSPS protein revealed no significant similarities to known toxic proteins.

5.1.3. Allergenicity[35]

The strategies to assess the potential allergenic risk are focused on the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified plant.

5.1.3.1. Assessment of allergenicity of the newly expressed protein

A weight-of-evidence approach was followed, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a).

The CP4 *epsps* gene originates from *Agrobacterium* sp. CP4, a soil microorganism that is not known to be allergenic.

Updated bioinformatic analyses[36] of the amino acid sequences of the CP4 EPSPS protein, using the criterion of 35 % identity in a window of 80 amino acids, revealed no significant similarities to known allergens. In addition, the applicant also performed analyses searching for matches of eight contiguous identical amino acid sequences between the CP4 EPSPS protein and known allergens, which confirmed the outcome of the previous bioinformatic analysis.

The studies on resistance to degradation of the CP4 EPSPS protein by proteolytic enzymes presented in the current application have been previously risk assessed by the EFSA GMO Panel (EFSA, 2008; EFSA GMO Panel 2012a, b).

The EFSA GMO Panel has previously evaluated the safety of the CP4 EPSPS protein in the context of several other applications and no concerns on allergenicity were identified (e.g. EFSA, 2006, 2008;

[32] Part II Scientific information. Annex: Bhatka (2010).
[33] Part II Scientific information, Section A.4.2.1.
[34] Additional information: 19/12/2013.
[35] Part II Scientific information, Section A.5.
[36] Additional information: 19/12/ 2013.

EFSA GMO Panel 2012b). No concerns regarding adjuvanticity of this newly expressed protein were identified in the scientific literature or in the bioinformatics analyses.

The EFSA GMO Panel considers that there are no indications that the newly expressed CP4 EPSPS protein in oilseed rape MON 88302 may be allergenic in the intended conditions of use.

5.1.3.2. Assessment of allergenicity of the whole GM plant

Oilseed rape is not considered to be a common allergenic food (EC, 2007; OECD, 2011). Cases of occupational allergy to inhaled dust/flour (Monsalve et al., 1997; Suh et al., 1998) and of sensitisation and allergic reactions to seed (Poikonen et al., 2006; Puumalainen et al., 2006) and pollen (Chardin et al., 2008) have been reported. However, the main oilseed rape product in human food is rapeseed oil, which is highly purified and contains very low or negligible levels of proteins.

The EFSA GMO Panel considers that there is no evidence that the genetic modification could significantly change the overall allergenicity of oilseed rape MON 88302.

5.1.4. Nutritional assessment of GM food/feed[37]

The intended trait of oilseed rape MON 88302 is herbicide tolerance, with no intention to alter the nutritional parameters. As relevant compositional differences were not observed for oilseed rape MON 88302 (see Section 4.1.2), the nutritional value of food and feed derived from oilseed rape MON 88302 is not expected to differ from that of food and feed derived from non-GM oilseed rape varieties.

5.1.5. Post-market monitoring of GM food/feed

No indication that oilseed rape MON 88302 is any less safe than its conventional counterpart was identified during the risk assessment. Therefore, and in line with its guidelines (EFSA GMO Panel, 2011a), the EFSA GMO Panel considers that post-market monitoring of the GM food/feed is unnecessary.

5.2. Conclusion

The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly expressed CP4 EPSPS protein, and found no evidence that the genetic modification might significantly change the overall allergenicity of oilseed rape MON 88302. As relevant compositional differences were not observed for oilseed rape MON 88302, the nutritional value of food and feed derived from oilseed rape MON 88302 is not expected to differ from that of food and feed derived from non-GM oilseed rape varieties.

The EFSA GMO Panel concludes that oilseed rape MON 88302 assessed in this application is as safe and nutritious as its conventional counterpart and the non-GM oilseed rape reference varieties tested. In addition, the EFSA GMO Panel found no indication that the introduction of the event MON 88302 into other oilseed rape varieties would affect its safety with respect to potential effects on human and animal health.

6. Environmental risk assessment and monitoring plan

6.1. Evaluation of relevant scientific data

The scope of application EFSA-GMO-BE-2011-101 is for food and feed uses, import and processing, and does not include cultivation. Therefore, the environmental risk assessment is concerned with (1) the exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals fed GM material and those present in environments exposed to faecal material (manure and faeces); and (2) the

[37] Part II Scientific information, Section A.6.

accidental release into the environment of viable oilseed rape MON 88302 seeds (i.e. during transport and/or processing).

6.1.1. Environmental risk assessment[38]

6.1.1.1. Potential unintended effects on plant fitness due to the genetic modification[39]

The applicant presented agronomic and phenotypic data on oilseed rape MON 88302 gathered from field trials conducted in oilseed rape-growing areas in North America (two locations in the USA, three locations in Canada) during the 2009 growing season and in South America (four locations in Chile) during the 2009/2010 growing season (see Section 4). Seed and pollen characteristics of oilseed rape MON 88302 were also evaluated under growth chamber conditions.

The EFSA GMO Panel is of the opinion that the likely effect of the magnitude of the observed difference in days-to-first flowering between oilseed rape MON 88302 and the conventional counterpart on the potential of oilseed rape MON 88302 plants to exhibit increased survival, establishment and fitness is negligible and will thus not lead to any relevant increase in persistence or invasiveness. Oilseed rape varieties differ slightly in flowering time, and the reported values for oilseed rape MON 88302 are within the range of those reported in conventional oilseed rape. Moreover, feral oilseed rape populations generally consist of a mixture of different (including spring- and winter-sown) varieties (Pascher et al., 2010), varying in morphology and phenology, with seedlings emerging and flowering at various rates and times in the season (Crawley et al., 1993; Claessen et al., 2005a, b; Knispel and McLachlan, 2009). The observed difference in days-to-first flowering is unlikely to have a significant effect on population dynamics, compared with the main physiological differences between oilseed rape varieties in variables such as secondary dormancy, persistence in the seedbank, spread of emergence, resistance to grazing by slugs and snails, seasonality and propensity for seed drop, in addition to any unquantified differences in tolerance to roadside salt and pollution.

Although scientific uncertainty remains on the exact cause of the observed difference in days-to-first flowering, the dataset does not show major changes in phenotypic plant characteristics that indicate altered fitness, persistence and invasiveness of oilseed rape MON 88302 plants. It is therefore considered unlikely that oilseed rape MON 88302 plants will have a selective advantage for the genetic modification, unless they are exposed to glyphosate-based herbicides.

In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report of increased spread and establishment of oilseed rape MON 88302 or oilseed rape with comparable properties for the specific scope of this application, or of any change in survival capacity. The evidence on fitness, persistence and invasiveness of feral GMHT oilseed rape is derived from the following sources: (1) transplant or seed-sowing experiments; (2) ecophysiological experiments and models on comparative fitness; and (3) demographic studies and surveys to see whether feral oilseed rape invades natural habitats (Devos et al., 2012; COGEM, 2013).

- Field studies (source type 1, above) have confirmed that herbicide tolerance traits in oilseed rape do not confer a fitness advantage, unless the herbicides for which tolerance is obtained are applied. In these studies, the invasive potential of GM plants was assessed directly by releasing them into natural habitats and by monitoring their fitness in the subsequent generation(s). GMHT oilseed rape introduced into 12 different habitats at three sites across the UK failed to persist in established vegetation: in none of the natural plant communities considered, was oilseed rape found after three years, even when vegetation had been removed in the first year of sowing (Crawley et al., 1993, 2001). These experiments demonstrated that the genetic modification *per se* does not enhance ecological fitness (Hails et al., 1997; Hails and Morley, 2005).

[38] Part II Scientific information, Section E.
[39] Part II Scientific information, Sections E.2.2 and E.3.1.

– Experiments and models on fitness differences between the GM plant and its non-GM counterpart (source type 2, above) are usually inferred from a composite measure of relative plant germination, emergence, growth, survivorship, biomass and fecundity (Fredshavn et al., 1995; Warwick et al., 1999, 2004, 2009; Norris and Sweet, 2002; Claessen et al., 2005a, b; Simard et al., 2005; Garnier and Lecomte, 2006; Garnier et al., 2006; Londo et al., 2010, 2011; Watrud et al., 2011). Beckie et al. (2004) showed that GMHT oilseed rape with single or multiple herbicide tolerance traits is not more persistent (weedier) than non-GMHT plants. Additionally, greenhouse studies, in which the fitness of oilseed rape volunteers with no, single or multiple herbicide tolerance was assessed, have shown no or little difference in fitness among oilseed rape plants in the absence of herbicide pressure (Simard et al., 2005). There is also no evidence that tolerance to the herbicidal active substance glyphosate enhances seed dormancy, and thus the persistence of GMHT oilseed rape plants, compared with the conventional counterpart (Hails et al., 1997; Sweet et al., 2004; Lutman et al., 2005, 2008; Messéan et al., 2007). Seed dormancy is more likely to be affected by the genetic background of parental genotypes than the acquisition of herbicide tolerance traits (López-Granados and Lutman, 1998; Lutman et al., 2003; Gruber et al., 2004; Gulden et al., 2004a, b; Messéan et al., 2007; Baker and Preston, 2008; de Jong et al., 2013; Schatzki et al., 2013).

– Demographic studies and surveys (source type 3, above) have shown the ability of oilseed rape to establish self-perpetuating populations outside agricultural areas, mainly in semi-natural and ruderal habitats in different countries (reviewed by Devos et al., 2012; Bauer-Panskus et al., 2013; COGEM, 2013; Hecht et al., 2014), but overall revealed that feral oilseed rape is confined to ruderal habitats and that feral GMHT oilseed rape does not behave as an ecologically hazardous invasive species (see Appendix D and references therein of EFSA, 2013b, c). Oilseed rape is generally regarded as an opportunistic species, which can take advantage of disturbed sites due to its potential to germinate and capture resources rapidly. In undisturbed natural habitats, oilseed rape lacks the ability to establish stable populations, possibly due to the absence of competition-free germination sites (Crawley et al., 1993, 2001; Warwick et al., 1999; Hails et al., 2006; Sutherland et al., 2006; Damgaard and Kjaer, 2009). Moreover, in controlled sowings into road verges, field margins and wasteland, very few seedlings survived to maturity due to grazing (e.g. by molluscs) and abiotic stress (Charters et al., 1999). Kos et al. (2012) postulated that the low glucosinolate content in current oilseed rape varieties renders the plants more susceptible to leaf and seed herbivory, reducing seed production (see also COGEM, 2013).

  Once established in competition-free germination sites, feral populations decline over a period of years. A 10-year survey (1993–2002), along road verges of a motorway revealed that most quadrats showed transient populations lasting one to four years (Crawley and Brown, 2004). These data and those from other demographic studies indicate a substantial turnover of populations of feral oilseed rape: only a small percentage of populations occur at the same location over successive years, but the majority of plants did not survive, resulting in rapidly declining populations (Crawley and Brown, 1995, 2004; Charters et al., 1999; Peltzer et al., 2008; Elling et al., 2009; Knispel and McLachlan, 2009; Nishizawa et al., 2009; Mizuguti et al., 2011; Squire et al., 2011). However, if habitats are disturbed on a regular basis by anthropogenic activities such as mowing, herbicide applications or soil disturbance, or natural occurrences such as flooding, then feral populations can persist for longer periods (Claessen et al., 2005a; Garnier et al., 2006).

  The persistence or recurrence of a population in one location is variously attributed to replenishment with fresh seed spills, to recruitment from seed emerging from the soil seedbank or shed by resident feral adult plants, or to redistribution of feral seed from one location to another. Although many feral populations observed over multiple years were transient at a local scale (e.g. Crawley and Brown, 1995, 2004; Knispel et al., 2008), this apparent transience is probably counterbalanced at a landscape scale by repeated seed addition and redistribution from various sources. Local declines or extinctions in above-ground feral

populations are likely to be temporary and asynchronous at large spatial scales (Charters et al., 1999; Crawley and Brown, 2004; Peltzer et al., 2008; Knispel and McLachlan, 2009; Nishizawa et al., 2009). On a larger scale in the landscape, feral oilseed rape can thus be considered long lived, with a proportion of the populations founded by repeated fresh seed spills from both agricultural fields and transport, and the remainder resulting from the continuous recruitment of seeds from local feral soil seedbanks (Pivard et al., 2008a, b).

The evidence described above indicates that GMHT oilseed rape, including oilseed rape MON 88302, is neither more likely to survive, nor to be more persistent or invasive, than its conventional counterpart, unless the plants are exposed to glyphosate-based herbicides. The ability of oilseed rape to successfully invade ruderal habitats appears to be limited principally by the availability of seed germination sites and low interspecific plant competition, and there is no evidence that genes conferring herbicide tolerance significantly alter its competitive ability (Kos et al., 2012). Moreover, in controlled sowings into road verges, field margins and wasteland, very few seedlings survived to maturity due to grazing (e.g. by molluscs), plant competition and abiotic stress (Charters et al., 1999).

Glyphosate-based herbicides are frequently used for the control of vegetation along railway tracks, on arable land, in open spaces, on pavements or in industrial sites (Monsanto, 2010). In these areas, the glyphosate tolerance trait is likely to increase the fitness of GMHT plants (be it feral plants or progeny from hybrids of oilseed rape and wild relatives) relative to non-glyphosate-tolerant plants when exposed to glyphosate-based herbicides (Londo et al., 2010, 2011; Watrud et al., 2011). The occurrence of feral GMHT oilseed rape resulting from seed import spills is likely to be low under an import scenario (Devos et al., 2012). Therefore, feral oilseed rape plants would not create additional agronomic or environmental impacts, even after exposure to glyphosate-based herbicides (Kim, 2012).

The accidental release of oilseed rape MON 88302 seeds (i.e. during transport and/or processing) would not result in the establishment of plants exhibiting dissemination capabilities any different from those of existing conventional oilseed rape varieties and would not create any additional agronomic or environmental impacts. Therefore, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the accidental release into the environment of oilseed rape MON 88302 will not be different from that of conventional oilseed rape varieties. The EFSA GMO Panel considers that, in the context of intended uses, the differences observed are unlikely to significantly affect the overall fitness, invasiveness or weediness of the oilseed rape MON 88302.

The EFSA GMO Panel concludes that, considering the scope of this application, the available data and the limited ability of oilseed rape to survive outside cultivated land (which is confined to ruderal habitats), the likelihood of any additional effects owing to the accidental release into the environment of viable seeds from oilseed rape MON 88302 is very low.

6.1.1.2. Potential for gene transfer[40]

The EFSA GMO Panel evaluated the potential for horizontal and vertical gene flow of oilseed rape MON 88302, as well as the potential environmental consequences of such gene transfer. A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, through either horizontal gene transfer of DNA or vertical gene flow via the dispersal of pollen and seeds.

(a) Plant-to-bacteria gene transfer[41]

Genomic plant DNA is a component of several food and feed products derived from oilseed rape. It is well documented that DNA present in food and feed becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments of ingested DNA, including the recombinant fraction of such DNA, to bacteria

[40] Part II Scientific information, Sections E.2.1, E.3.1 and E.3.2.
[41] Part II Scientific information, Section E.3.2.

in the digestive tract of humans, domesticated animals and other environments exposed to the GM plant or plant material is expected.

Current scientific knowledge of recombination processes in bacteria suggests that horizontal transfer of non-mobile, chromosomally located DNA fragments between unrelated organisms (such as from plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (for further details, see EFSA, 2009b).

A successful horizontal gene transfer would require stable insertion of the recombinant DNA sequences into a bacterial genome and a selective advantage to be conferred on the transformed host. The only mechanism known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes is HR. The similarity between the plant and bacterial sequences can be situated in the transgene itself or in the flanking regions. In the case of sequence identity with the transgene itself, recombination would result in gene replacement. In the case of identity with the flanking regions of the transgene, recombination could result in the insertion of additional DNA sequences in bacteria.

Bioinformatic analysis of the inserted DNA and flanking regions (Section 3.1.2) did not identify sufficient sequence identity with bacterial DNA (including the modified CP4 *epsps* gene, which has been codon-optimised for expression in plants) that would facilitate HR-mediated gene transfer between plants and bacteria[42].

In addition to homology-based recombination processes, non-homologous (illegitimate) recombination that does not require similarity between the recombining DNA molecules is theoretically possible. However, illegitimate recombination events have not been detected in studies that have exposed bacteria to high concentrations of GM plant DNA (see EFSA, 2009b). Thus, these processes are not considered to occur at levels that would make them relevant in assessments of plant-to-bacteria gene transfer events.

In conclusion, the EFSA GMO Panel did not identify properties of the DNA inserted into oilseed rape MON 88302 that would change its likelihood of horizontal transfer compared with other plant genes. Therefore, the EFSA GMO Panel concludes that the recombinant DNA in oilseed rape MON 88302 does not represent an environmental risk in relation to its potential for horizontal transfer to bacteria.

(b) Plant-to-plant gene transfer[43]

Oilseed rape is an open-pollinating crop plant capable of cross-pollinating with other *Brassica* crops (Eastham and Sweet, 2004) and some wild relatives (Devos et al., 2009). It produces large amounts of small seeds, which can survive and persist for many years in soil (Lutman et al., 2004, 2005, 2008; Begg et al., 2006; Messéan et al., 2007; D'Hertefeldt et al., 2008; Gruber et al., 2008; Andersen et al., 2010; Beckie and Warwick, 2010; Munier et al., 2012) and which tend to be widely dispersed during farm and transport operations (Price et al., 1996; Zwaenepoel et al., 2006; von der Lippe and Kowarik, 2007b; Pivard et al., 2008a, b; Bailleul et al., 2012; Allnutt et al., 2013). Seed dispersal results in oilseed rape being a major weed (volunteer) in crop rotations and the occurrence of feral plants outside cultivated areas, often in ruderal—non-cropped, disturbed—habitats, where they can survive and reproduce successfully without management (Gressel, 2005; Bagavathiannen and Van Acker, 2008). In areas where oilseed rape is cultivated, feral oilseed rape typically originates from the spillage of seeds during its transport to and from fields and to processing plants, the redistribution of seeds by field equipment and grain trailers (Bailleul et al., 2012; Allnutt et al., 2013) or from the dispersal of seeds, for example by birds and mammals (von der Lippe and Kowarik, 2007a, b; Wichmann et al., 2009). The transport of seeds following both cultivation and importation has resulted in dispersal of seeds into a range of environments. Volunteer populations in agricultural fields arise mostly from seeds lost through the shattering of the seed-bearing pods before and during harvest. At seed maturity,

[42] Additional information: 03/03/2014 (including Yan and Silvanovich (2014)).
[43] Part II Scientific information, Sections E.2.1 and E.3.1.

the pods become fragile and easily split open, resulting in losses that can reach up to 10 % of the seed yield (Thomas et al., 1991; Price et al., 1996; Morgan et al., 1998; Hobson and Bruce, 2002; Gulden et al., 2003).

Oilseed rape is an outcrossing species with potential to cross-pollinate other oilseed rape types with varying frequency depending on flowering synchrony, spatial arrangement of plants, presence of pollinator insects and other factors as reviewed by Eastham and Sweet (2004) (see also Hüsken and Dietz-Pfeilstetter, 2007; Beckie and Hall, 2008; Devos et al., 2009; Zhao et al., 2013; Stanley et al., 2014). Feral oilseed rape MON 88302 plants arising from spilled seeds could therefore pollinate crop plants of non-GM oilseed rape if feral populations are immediately adjacent to field crops (Garnier and Lecomte, 2006). Shed seeds from cross-pollinated crop plants could emerge as GM volunteers in subsequent crops. Squire et al. (2011) and Devos et al. (2012) considered that the frequency of such events was likely to be extremely low and concluded that this route of gene flow would not introduce significant numbers of GM plants into farmland or result in any environmental consequences. The coexistence between GM and non-GM crops is not in the remit of the EFSA GMO Panel and therefore is not considered further here.

Oilseed rape is known to spontaneously hybridise with some sexually compatible wild relatives (Scheffler and Dale, 1994; Ellstrand et al., 1999, 2013; Devos et al., 2009; Andersson and de Vicente, 2010; Liu et al., 2010, 2012, 2013; Huangfu et al., 2011; Tsuda et al., 2011; de Jong and Hesse, 2012, Luijten et al., 2014). Several oilseed rape × wild relative hybrids have been reported in the scientific literature, but, under field conditions, transgene introgression has only been confirmed for progeny of oilseed rape × *Brassica rapa* hybrids (Hansen et al., 2001, 2003; Warwick et al., 2003, 2008; Norris et al., 2004; Jørgensen, 2007; Ellstrand et al., 2013). Owing to ecological and genetic barriers, not all relatives of oilseed rape share the same potential for hybridisation and transgene introgression (Jenczewski et al., 2003; Chèvre et al., 2004; FitzJohn et al., 2007; Wilkinson and Ford, 2007; Devos et al., 2009; Jørgensen et al., 2009; Luijten and de Jong, 2011; Liu et al., 2013). For transgene introgression to occur, both species must occur in their respective distribution range of viable pollen. This requires at least a partial overlap in flowering in time and space, and sharing of common pollinators (if insect pollinated) (Pascher et al., 1999, 2011; Wilkinson et al., 2000, 2003a; Chèvre et al., 2004; Simard and Légère, 2004; Allainguillaume et al., 2006; Simard et al., 2006; Wurbs et al., 2010; Liu et al., 2013; Luijten et al., 2014; Ohigashi et al., 2014). Sufficient level of genetic and structural relatedness between the genomes of both species also is needed to produce viable and fertile oilseed rape × wild relative hybrids that stably express the transgene (e.g. Heyn, 1977; Kerlan et al., 1993). Genes, subsequently, must be transmitted through successive backcross generations or selfing, so that the transgene becomes stabilised into the genome of the recipient (de Jong and Rong, 2013). As no or only very low numbers of viable and fertile hybrids are obtained between oilseed rape and most of its wild relatives under ideal experimental conditions (e.g. through the use of artificial pollination and embryo rescue techniques in laboratory conditions (see FitzJohn et al., 2007)), Wilkinson et al. (2003b) concluded that exposure under real conditions is likely to be negligible, and the probability of transgene introgression is extremely small in most instances, with the exception of *B. rapa* in areas where it occurs close to oilseed rape (Vacher et al., 2011). Transgene introgression is likely to take place when oilseed rape and *B. rapa* grow in close proximity over successive growing seasons, especially if no significant fitness costs are imposed to backcross plants by transgene acquisition (Snow et al., 1999). However, hybrids between *B. napus* and *B. rapa* are mostly triploid with low male fertility, and hence have a low ability to pollinate and form backcrosses with *B. napus* (Norris et al., 2004). Incidences of hybrids and backcrosses with *B. rapa* were found to be low in fields in Denmark (Jørgensen et al., 2004) and the UK (Norris et al., 2004). Recent observations in Canada confirmed the persistence of a glyphosate tolerance trait over a period of six years in a population of *B. rapa* in the absence of herbicide pressure (with the exception of possible exposure to the herbicidal active substance glyphosate in one year) and in spite of fitness costs associated with hybridisation (Warwick et al., 2008). A single GM *B. rapa* × *B. napus* hybrid was also reported along a road in Vancouver (Yoshimura et al., 2006), confirming the hybridisation possibility between these two *Brassica* species, albeit at very low frequencies. Elling et al. (2009) also described the detection of triploid hybrid offspring with intermediate morphology and oilseed rape microsatellite alleles from a single *B. rapa*

mother plant. However, Elling et al. (2009) measured the extent of hybridisation between autotetraploid *B. rapa* varieties (female) and *B. napus* (pollen donor) under experimental field conditions, and found that hybridisation with tetraploid *B. rapa* seemed to be more likely than with diploid *B. rapa*. They reported that male fertility was higher in these hybrids than those formed with diploid *B. rapa* and suggested that introgression frequencies from *B. napus* to *B. rapa* would be higher in tetraploid *B. rapa*. Elling et al. (2009) also reported the presence of some feral tetraploid *B. rapa* populations in northwest Germany, but did not report on interspecific hybrids or backcrosses in these populations. Findings reported by Luijten et al. (2014) suggest that barriers may limit introgression between oilseed rape × *B. rapa* and that crop genes may not necessarily remain present in wild populations in the long run.

Surveys and analyses conducted in Japan monitored transgenes in seed collected from populations of wild relatives (*B. rapa* and *B. juncea*) sampled at several ports and along roadsides and riverbanks. Transgenes were detected in only two hybrid plants derived from a cross between *B. napus* and *B. rapa* (Saji et al., 2005; Aono et al., 2006, 2011). There have been very few other attempts to measure the transfer of genetic material from feral plants to wild relatives. Introgression of genetic material from feral oilseed rape to wild relatives, while theoretically possible, is likely to be very low due to the combined probabilities of spillage of GMHT oilseed rape in areas where wild relatives (e.g. *B. rapa*) are present, germination, survival of oilseed rape plants, hybridisation with its wild relatives, survival and the low fertility of interspecific hybrids restricting backcrossing with wild relatives.

There is no evidence to suggest that herbicide tolerance traits in a wild relative changes the plant's behaviour (Scheffler and Dale, 1994; Eastham and Sweet, 2002; Warwick et al., 2003, 2004, 2008; Chèvre et al., 2004; Jørgensen, 2007; Jørgensen et al., 2009), or the scale and nature of its interactions with associated flora and fauna (Wilkinson et al., 2003b; Wilkinson and Ford, 2007). Progeny from hybrids of oilseed rape and wild relatives that bear the herbicide tolerance trait do not show any enhanced fitness, persistence and invasiveness, and behave in the same way as their conventional counterparts, unless the herbicides to which tolerance is obtained are applied (Londo et al., 2010, 2011; Watrud et al., 2011).

Glyphosate-based herbicides are frequently used for the control of vegetation along railway tracks, on arable land, in open spaces, on pavements or in industrial sites (Monsanto, 2010). In these areas, the glyphosate tolerance trait is likely to increase the fitness of GMHT plants (be it feral plants or progeny from hybrids of oilseed rape and wild relatives) relative to non-glyphosate-tolerant plants when exposed to glyphosate-based herbicides (Londo et al., 2010, 2011; Watrud et al., 2011). However, both the occurrence of feral GMHT oilseed rape resulting from seed import spills and the introgression of genetic material from feral oilseed rape to wild relatives are likely to be low under an import scenario. Therefore, feral oilseed rape plants and genes introgressed into other cross-compatible plants would probably not create any additional agronomic or environmental impacts, even after exposure to glyphosate-based herbicides (Kim, 2012).

The EFSA GMO Panel confirms that feral GMHT oilseed rape plants are likely to occur wherever GMHT oilseed rape is transported. However, there is no evidence that the herbicide tolerance trait results in enhanced fitness, persistence or invasiveness of oilseed rape MON 88302, or hybridising wild relatives, unless these plants are exposed to glyphosate-based herbicides. Escaped oilseed rape plants and genes introgressed into other cross-compatible plants would therefore not create any additional agronomic or environmental impacts.

In conclusion, as oilseed rape MON 88302 or hybridising wild relatives have no altered survival, multiplication or dissemination characteristics, except when exposed to glyphosate-based herbicides, the EFSA GMO Panel considers that the likelihood of unintended environmental effects as a consequence of the spread of genes from this GM oilseed rape in Europe will not differ from that of conventional oilseed rape varieties.

The EFSA GMO Panel does not consider the occurrence of occasional feral oilseed rape MON 88302 plants, pollen dispersal and consequent cross-pollination as environmental harm in itself, and is primarily concerned with assessing the environmental consequences of this occurrence on biotic interactions and ecosystems. However, should risk managers consider the control of feral oilseed rape plants desirable, the EFSA GMO Panel recommends implementing appropriate communication means for the timely reporting of control failures of feral oilseed rape populations.

6.1.1.3. Interactions of the genetically modified plant with target organisms[44]

Interactions of oilseed rape MON 88302 with target organisms are not considered to be a relevant issue by the EFSA GMO Panel, as there are no target organisms.

6.1.1.4. Interactions of the genetically modified plant with non-target organisms[45]

Owing to the intended uses of oilseed rape MON 88302, which exclude cultivation, and because of the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms are not considered to be a relevant issue by the EFSA GMO Panel. In addition, there are no indications that the expression of the CP4 EPSPS protein in glyphosate-tolerant plants causes direct adverse effects on non-target organisms (CERA, 2010).

6.1.1.5. Potential interactions with the abiotic environment and biochemical cycles[46]

Given the scope of this application, which excludes cultivation of oilseed rape MON 88302, and the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles are not considered to be a relevant issue by the EFSA GMO Panel.

6.1.2. Post-market environmental monitoring[47]

The objectives of a post-market environmental monitoring plan according to Annex VII of Directive 2001/18/EC are (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct; and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the environmental risk assessment.

Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific content of the post-market environmental monitoring plan provided by the applicant (EFSA GMO Panel, 2011b).

The potential exposure to the environment of oilseed rape MON 88302 would be through ingestion by animals and their faecal material leading to exposure of the gastrointestinal tract and soil microbial populations to recombinant DNA, and through the accidental release into the environment of GM oilseed rape seeds during transport and/or processing. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of oilseed rape MON 88302. As the environmental risk assessment does not cover cultivation and identified no potential adverse environmental effects, no case-specific monitoring is necessary.

The post-market environmental monitoring plan proposed by the applicant includes: (1) the description of an approach involving operators (federations involved in oilseed rape import and processing) reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system newly established by EuropaBio for the collection of the information recorded by the various operators; and (3) the use of networks of existing surveillance systems (Lecoq et al., 2007; Windels et al., 2008). The applicant

[44] Part II Scientific information, Section E.3.3.
[45] Part II Scientific information, Section E.3.4.
[46] Part II Scientific information, Section E.3.6.
[47] Part II Scientific information, Section E.4.

proposes to submit a post-market environmental monitoring report on an annual basis and a final report at the end of the consent period.

The EFSA GMO Panel considers the scope of the post-market environmental monitoring plan provided by the applicant consistent with the intended uses of oilseed rape MON 88302, as the environmental risk assessment does not cover cultivation and identified no potential adverse environmental effects. In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in the case of accidental release of viable seeds of oilseed rape MON 88302. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the post-market environmental monitoring plan.

6.2. Conclusion

In the case of accidental release into the environment of viable oilseed rape MON 88302 seeds, there are no indications of an increased likelihood of spread and establishment of feral oilseed rape MON 88302 plants or hybridising wild relatives, unless these plants are exposed to glyphosate-based herbicides. Given the scope of this application, only low-level exposure of environmental bacteria, including those in the gastrointestinal tract, is expected. Risks associated with an unlikely, but theoretically possible, horizontal gene transfer from oilseed rape MON 88302 to bacteria have not been identified. Considering the scope of this application, the risk to non-target organisms is extremely low owing to the expected confined occurrence of feral oilseed rape plants to ruderal habitats and the low levels of exposure through other routes. In addition, there are no indications that the expression of the CP4 EPSPS protein in glyphosate-tolerant plants causes direct adverse effects on non-target organisms. Interactions with the biotic and abiotic environment are therefore not considered to be a relevant issue.

The scope of the post-market environmental monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of oilseed rape MON 88302 and the EFSA GMO Panel guidelines on the post-market environmental monitoring of GM plants. In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable seeds of oilseed rape MON 88302.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was asked to carry out a scientific assessment of oilseed rape MON 88302 for import, processing, and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data provided for oilseed rape MON 88302 did not raise safety issues.

Based on the agronomic and phenotypic characteristics of oilseed rape MON 88302 tested under field conditions, no biologically relevant differences were observed between oilseed rape MON 88302 and its conventional counterpart, except for days-to-first flowering. The observed difference for days-to-first flowering could be attributed to either the variability in the genetic background of the Ebony population or an unintended effect due to the genetic transformation process. No biologically relevant differences were identified in the compositional characteristics of seeds obtained from oilseed rape MON 88302 that would require further assessment with regard to safety.

The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly expressed CP4 EPSPS protein, and found no evidence that the genetic modification might significantly change the overall allergenicity of oilseed rape MON 88302. As relevant compositional differences were not observed for oilseed rape MON 88302, the nutritional value of food and feed derived from oilseed rape MON 88302 is not expected to differ from that of food and feed derived from non-GM oilseed rape varieties.

The EFSA GMO Panel concludes that oilseed rape MON 88302, assessed in this application, is as safe and nutritious as its conventional counterpart and the non-GM oilseed rape reference varieties tested. In addition, the EFSA GMO Panel found no indication that the introduction of the event MON 88302 into other oilseed rape varieties would affect its safety with respect to potential effects on human and animal health.

In the case of accidental release into the environment of viable seeds of oilseed rape MON 88302, there are no indications of an increased likelihood of spread and establishment of feral oilseed rape MON 88302 plants or hybridising wild relatives, unless these plants are exposed to glyphosate-based herbicides. The likely effect of the magnitude of the observed difference in days-to-first flowering between oilseed rape MON 88302 and the conventional counterpart on the potential of oilseed rape MON 88302 plants to exhibit increased survival, establishment and fitness is negligible and will thus not lead to any relevant increase in persistence or invasiveness. Considering the scope of this application, interactions of oilseed rape MON 88302 with the biotic and abiotic environment are not considered to be a relevant issue. Bioinformatic analysis of the inserted DNA and flanking regions did not identify sufficient sequence identity with bacterial DNA (including the modified CP4 *epsps* gene, which has been codon-optimised for expression in plants) that would facilitate homologous recombination-mediated gene transfer between plants and bacteria. Therefore, risks associated with an unlikely, but theoretically possible, horizontal transfer of recombinant genes from oilseed rape MON 88302 to bacteria have not been identified. The scope of the post-market environmental monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of oilseed rape MON 88302 and the EFSA GMO Panel guidelines on the post-market environmental monitoring of GM plants. In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable seeds of oilseed rape MON 88302.

In conclusion, the EFSA GMO Panel considers that the information available for oilseed rape MON 88302 addresses the scientific issues indicated by the guidelines of the EFSA GMO Panel and the scientific comments raised by the Member States, and that oilseed rape MON 88302 is as safe as its conventional counterpart and other non-GM oilseed rape varieties, and is unlikely to have adverse effects on human and animal health and the environment in the context of the scope of this application.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of Belgium, received 8 September 2011, concerning a request for placing on the market of oilseed rape MON 88302 (application reference EFSA-GMO-BE-2011-101) in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 5 October 2011, from EFSA to the Competent Authority of Belgium.

3. Letter from EFSA to applicant, dated 19 October 2011, requesting additional information under completeness check.

4. Letter from applicant to EFSA, received 13 February 2012, providing additional information under completeness check.

5. Letter from EFSA to applicant, dated 2 March 2012, requesting additional information under completeness check.

6. Letter from applicant to EFSA, received 13 March 2012, providing additional information under completeness check.

7. Letter from EFSA to applicant, dated 2 April 2012, delivering the 'Statement of Validity' of application EFSA-GMO-BE-2011-101, oilseed rape MON 88302 submitted by Monsanto under Regulation (EC) No 1829/2003.

8. Letter from EFSA to applicant, dated 24 April 2012, requesting additional information and stopping the clock on behalf of the EURL-GMFF.

9. Letter from EFSA to applicant, dated 1 June 2012, re-starting the clock upon submission of the requested information to the EURL-GMFF.

10. Letter from EFSA to applicant, dated 20 July 2012, requesting additional information and stopping the clock on behalf of the EURL-GMFF.

11. Letter from applicant to EFSA, dated 11 September 2012, requesting clarifications.

12. Letter from EFSA to applicant, dated 13 September 2012, requesting additional information and maintaining the clock stopped.

13. Letter from applicant to EFSA, received 10 October 2012, providing additional information.

14. Letter from EFSA to applicant, dated 30 October 2012, re-starting the clock for the EUR-GMFF and maintaining the clock stopped for EFSA.

15. Letter from EFSA to applicant, dated 30 October 2012, requesting additional information and stopping the clock on behalf of the EURL-GMFF and maintaining the clock stopped for EFSA.

16. Letter from EFSA to applicant, dated 16 January 2013, re-starting the clock for EURL-GMFF and maintaining the clock stopped for EFSA.

17. Letter from EFSA to applicant, dated 30 January 2013, requesting additional information and maintaining the clock stopped.

18. Letter from applicant to EFSA, received 27 February 2013, providing additional information.

19. Letter from EFSA to applicant, dated 24 April 2013, re-starting the clock.

20. Letter from EFSA to applicant, dated 13 August 2013, requesting additional information and stopping the clock.

21. Letter from applicant to EFSA, dated 2 September 2013, providing additional information.

22. Letter from EFSA to applicant, dated 12 December 2013, requesting additional information and maintaining the clock stopped.

23. Letter from applicant to EFSA, received 19 December 2013, providing additional information spontaneously.

24. Letter from applicant to EFSA, dated 3 March 2014, providing additional information.

25. Letter from EFSA to applicant, dated 29 April 2014, re-starting the clock.

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